American Journal of Hematology 54:16–22 (1997)

Genetic Analysis of β-Thalassemia Intermedia in Israel: Diversity of Mechanisms and Unpredictability of Phenotype

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Molecular analysis was performed on 95 Israeli patients with thalassemia intermedia, representing 60 families of Arab (Moslem and Christian), Jewish, Druze, and Samaritan origin. There was a wide range of phenotypic severity, with baseline hemoglobin levels ranging from 5.5 to 10.7. Eighteen thalassemia mutations were found (29 genotypes), which were subdivided into groups, according to the severity of mutations. A consistently mild phenotype (10 families) was caused by compound heterozygosity for a silent mutation, such as −101 C-T or by coexistence of triplicated α-globin genes with thalassemia trait. In 39 thalassemia intermedia families, the genotype which was found was one which led to severe thalassemia intermedia, or, in other families, was associated with thalassemia major. Elevated hemoglobin F ameliorated the disease in some patients with a severe genotype. We did not find a beneficial effect of concurrent α-thalassemia in any of the families studied. In 11 families, only one β-thalassemia allele was identified. One was a dominant thalassemia intermedia allele. Three additional families with heterozygous β-thalassemia had excess α-globin genes (5 or 6 total). In 7 of these heterozygotes, no explanation was found for the thalassemia intermedia phenotype. Our results suggest a substantial influence of as yet unknown genetic modifiers. These findings have important implications for prenatal diagnosis and for the genetic counseling of families with thalassemia intermedia. Am. J. Hematol. 54:16–22, 1997 © 1997 Wiley-Liss, Inc.

Key words: thalassemia intermedia; genotype/phenotype; mutations; genetic modifiers; genetic counseling

INTRODUCTION

β-thalassemia is an autosomal recessive disorder which can be caused by any of nearly 180 mutations in the gene coding for the β-chain of the hemoglobin tetramer [1]. The disease causes anemia of variable severity which becomes manifest in early childhood. Most patients have the severe form of the disease referred to as thalassemia major, characterized by lifelong blood transfusion dependency. A smaller proportion of the patients have a milder form of the disease known as thalassemia intermedia, in which there is only moderate anemia, with minimal or no transfusion requirement. The clinical severity of thalassemia is influenced by the particular mutation(s) of the β-globin gene which are present in each patient, but is also known to be influenced by other factors which affect either α- and γ-globin expression.

In Israel, there are several hundred affected individuals with β-thalassemia. Approximately one fifth of those under medical supervision have thalassemia intermedia. We undertook to determine the spectrum of mutations of a large cohort of patients referred from all parts of the country, which represent the great majority of thalassemia intermedia patients in Israel.

Our laboratory is the center for DNA-based prenatal diagnosis of thalassemia in Israel. Because the spectrum of clinical severity of thalassemia intermedia is extremely

Contract grant sponsor: Chief Scientist's Office, Ministry of Health, Israel; Contract grant sponsor: United States–Israel Binational Science Foundation; Contract grant number: 91-00055; Contract grant sponsor: NIH; Contract grant number: 7R37 DK 13983-06; Contract grant sponsor: Rochlin Foundation.

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Received 19 January 1996; Accepted 7 August 1996

diverse, it was imperative to analyze the relationship between the genotype and the phenotype of thalassemia intermedia. Besides serving as a data base for genetic counseling, the information would also facilitate patient selection for novel therapeutic approaches, such as erythropoietin therapy, or bone marrow transplantation. Here we report the results of the analysis of the molecular basis of the disease in these patients, and the relationship of the genotypes to the phenotypes.

MATERIALS AND METHODS

Patient Referral

Patients were referred for analysis by treating physicians throughout Israel, for any of the following reasons: clarification of the diagnosis, considerations related to genetic counseling, or evaluation prior to referral for novel therapies.

Ninety-five patients from 60 families were analyzed. These represented 20 Jewish families (18 Kurdish, 1 North African, 1 Ashkenazi), 36 Arab families (27 Moslem, 9 Christian), 3 Samaritan families, and 1 Druze family. In 25 families, there was more than one patient (range 2–5). When possible, all sibs were examined and analyzed in parallel.

Clinical criteria for inclusion as thalassemia intermedia follow the general guidelines in the literature [2,3]. Since the disease spectrum of thalassemia is highly variable, we defined thalassemia intermedia using inclusion and exclusion criteria. In its mildest form, thalassemia intermedia was differentiated from the asymptomatic carrier state (thalassemia minor) by the presence of: severely pathological blood smear, significant splenomegaly, and anemia out of proportion to thalassemia minor (for males, Hb <12; for females, Hb <10). Iron deficiency and other potential causes for anemia and/or splenomegaly were ruled out. In many patients, organ disease such as bony abnormalities (including thalassemic facies and other deformities, characteristic of ineffective erythropoiesis and bone marrow hyperexpansion), endocrine disturbances, or other evidence of iron overload, were present. To distinguish from α-thalassemia, high HbA2 and/or HbF level $>1\%$ were required. To distinguish from thalassemia major, other criteria were used, which were: (1) Age at diagnosis over 2 years old. (2) Absence of prolonged transfusion dependency. (3) Good growth when transfusion independent, for at least a portion of early childhood.

Hematological data were obtained using standard laboratory techniques, as previously described [4].

DNA Analysis

DNA was isolated from peripheral blood using standard laboratory methods [5,6]. PCR was performed and samples were screened for mutations as previously described [7,8].

Haplotype analysis of the β-globin cluster was performed as described by Orkin et al. [9]. α-globin gene analysis was performed as previously described [10]. DNA with unknown mutant alleles was analyzed by sequencing the PCR products, from -166 nt 5′ to the cap site of the β-globin gene to 79 nt 3′ to the poly(A) site, excluding IVS2 nt 130–655.

RESULTS

Mutational Anlaysis

A total of 18 different β-thalassemia mutations were identified (Table I). These include null mutations (β^o) as well as those allowing reduced β-globin synthesis (β^+). Table I summarizes the mutations, and their ethnic distribution. Three of the mutations are silent β^+ mutations, 4 are moderate β^+ mutations, whereas the majority, 11, are severe β^+ or β^o mutations.

These mutations combined to result in a large number of genotypes (29 total), many of which were found only in a single family (Tables II, III). These results substantiate the inherent molecular heterogeneity of this group of patients. Some of this heterogeneity is attributable to the large number of ethnic groups in our population. Some of the mutations (such as -101 C-T) were found only in one ethnic group (Table I) while others were distributed among several ethnic groups.

Thalassemia Intermedia Patients Carrying Two β-Thalassemia Alleles

To enable analysis of phenotype-genotype correlations despite the large number of genotypes, we classified the genotypes into 5 groups based on severity of mutations (Groups I–V, Table II). These groups, as detailed below, result in strikingly different clinical phenotypes. Some genotypes were consistently mild and always resulted in thalassemia intermedia. Other genotypes were variable in severity and were found both in thalassemia intermedia patients as well as other patients with thalassemia major (Table II).

Group I: Compound heterozygosity for a silent mutation and a severe β^+ or β^o mutation (7 families). This group had the mildest phenotype. All patients had mild anemia with Hb levels no lower than 9 g%. None had ever been transfused, even during pregnancy. There was no or minimal thalassemic facies and no evidence of iron overload. Splenomegaly was mild to moderate (2 to 10 cm below the costal margin; none was splenectomized.) Hemoglobin F levels were mildly elevated (4.4–23%, mean 10.1%, equivalent to 0.4–2.4 g/dl of HbF, mean: 1.09 g).

Group II: Homozygosity for IVS1 nt 6 (T-C) (22 families). Patients in this group had a very variable clinical picture. At one extreme were 9 patients who had a baseline Hb as high as 10.6 g/dl, who were not splenecto-

TABLE I. Mutations in Israeli Thalassemia Intermedia Patients by Ethnic Group and Severity of Mutation*

	Number of chromosomes					
	MA	K/OJ	S	CA	D	Total
Mild mutations						
-101 C-T	–	5	–	–	–	5
-88 C-A	–	1	–	–	–	1
Knossos	1	–	–	–	–	1
Moderate mutations						
IVS1 nt 6 (T-C)	34	1	6	7	–	48
TATA (-28 A-C)	1	13	–	–	–	14
TATA (-30 T-A)	–	–	–	2	–	2
Poly(A) nt 6 (A-G)	–	5	–	–	–	5
Severe mutations						
IVS2 nt 1 (G-A)	6	2	–	–	2	10
Nonsense 37	1	–	–	–	–	1
Nonsense 39	1	3	–	1	–	5
Frameshift 44 (-C)	–	1	–	–	–	1
IVS1 nt 1 (G-A)	–	–	–	4	–	4
IVS1 nt 110 (G-A)	6	1	–	–	–	7
IVS2 nt 745 (C-G)	–	1	–	–	–	1
Frameshift 36/37 (-T)	–	1	–	–	–	1
Nonsense 15	1	–	–	–	–	1
IVS1 nt 5 (G-C)	–	1	–	–	–	1
Codon 114 CTG-CCG	–	1	–	–	–	1
Normal	–	1	–	3	–	4
Unknown[a]	3	3	–	1	–	7
Total						120

*MA: Moslem Arabs; K/OJ: Kurdish or other Jews; S: Samaritans; CA: Christian Arabs; D: Druze.
[a]Unknown chromosomes were sequenced and no mutation was found; however, the thalassemia phenotype is unexplained by the mutation on the opposite chromosome (see text).

TABLE II. Genotypes of Homozygous or Compound Heterozygous Israeli Thalassemia Intermedia Patients

Group	Chromosome 1	Chromosome 2	No. of families	Specific for intermedia
I	-101 C-T	β^+/β^o [FS44, FS 36/37, N39, IVS1 nt 5, IVS2 nt 745 (+ α-genes)]	5	Yes
	-88 C-A	N39	1	Yes
	Hb Knossos	IVS1 nt 110	1	Yes
II	IVS1 nt 6 T-C	IVS1 nt 6 T-C	22	Yes
III	-28 A-C	-28 A-C	4	No
		poly(A) nt 6	3	Yes
	-30 T-A	-30 T-A	1	Yes
IV	IVS1 nt 6 T-C	β^o (N39, IVS1 nt 1, IVS2 nt 1)	3	No
	-28 A-C	β^+/β^o (IVS1 nt 110, N37)	2	No
	poly(A) nt 6	β^o (N39, IVS2 nt 1)	2	No
V	IVS2 nt 1	IVS2 nt 1	1	No
	IVS2 nt 1	β^+ (IVS1 nt 110)	4	No

TABLE III. Genotypes of Israeli Thalassemia Intermedia Patients with Heterozygous β-Thalassemia

Chromosome 1	Chromosome 2	α genes	No. of families
β^o (N39, IVS1 nt 1)	Normal	αα/ααα, ααα/ααα	3
Codon 114 CTG-CCG	Normal	−α/αα	1
β^+ or β^o (IVS2 nt 1, N15, -28 A-C, IVS1 nt 110, IVS1 nt 6, IVS1 nt 1)	None found	Normal	7

mized and rarely (or never) transfused. Some of these individuals had normal growth, mild thalassemic facies with mild bony abnormalities, spontaneous (though usually delayed) puberty and even normal fertility without hormonal therapy. At the other extreme were 9 patients who had a baseline Hb level of 6–7 g/dl, and/or who were transfused infrequently albeit regularly (> every 2 months) despite splenectomy, who had severe growth delay, pronounced bony changes/thalassemic facies, absent puberty without hormonal manipulation, and who required chelation therapy for iron overload. Intermediate in severity were 15 patients with modest anemia (Hb 7–8 g/dl) which improved upon splenectomy, with variable secondary manifestations. Four additional individuals could not be classified due to insufficient clinical information.

It is of note that the severity of the disease among sibs could vary considerably. This disparity could not be attributed to differences in α-globin genotypes or variations in HbF levels. The HbF ranged from 6–38%, mean 13.7% (equivalent to 0.33–3.5 g/dl, mean 1.06 g). In fact, there was no consistent relationship between HbF level and total Hb in this group of patients.

Group III: Homozygosity or compound heterozygosity for two moderate mutations (8 families). Homozygotes for a mutation in the TATA box (−28 A-C or −30 T-A) all had a moderate phenotype. Most were splenectomized. Some of the nonsplenectomized individuals received occasional transfusions; all had impaired growth, severe bony disease, heart disease, and/or other evidence of iron overload. The four individuals who were compound heterozygotes for the −28 A-C mutation and another moderate mutation (poly(A) nt 6, A-G) had only moderate anemia, mild to moderate splenomegaly (none were splenectomized), no transfusion requirement, and minimal bony changes. The range of HbF levels in this group was highly variable, ranging from 8–64%, mean 32% (equivalent to 0.65–4.42 g/dl, mean 2.1 g).

Group IV: Compound heterozygosity for one moderate and one severe mutation (7 families). These patients had a moderately severe phenotype: moderately severe (but transfusion independent) anemia (Hb 5.5–8.8 g/dl), marked splenomegaly often requiring splenectomy, prominent bony changes including thalassemic facies, and growth retardation. The fetal hemoglobin levels ranged from 28 to 70%, mean 46% (equivalent to 1.5–4.1 g/dl, mean 3.35 g). There was a clear inverse relationship between the HbF level and the total hemoglobin level. Thus, the severity of the disease was attenuated in part by the high HbF levels.

Group V: Compound heterozygosity for two severe mutations (5 families). These individuals were all symptomatic, with thalassemic facies, moderately severe anemia, many requiring occasional (3–4 per year) transfusions. Splenomegaly was pronounced, and many patients were splenectomized. Some required regular chelation therapy due to objective evidence of iron overload despite minimal transfusion requirement. HbF levels were extremely high, ranging from 75–95%, mean 81% (5.2–8.33 g/dl, mean 6.49 g), which ameliorated the anemia. Despite this, their other symptoms were nevertheless moderately severe.

Thalassemia Intermedia With a Single β-Thalassemia Allele

One patient was found to be heterozygous for the missense mutation, codon 114 CTG-CCG. The family pedigree indicated that this mutation is dominant as has been reported [11]. The same mutation has also been reported in an Italian patient, in whom it has arisen de novo [12]. Both the previous reports and our studies found the mutation to confer a thalassemia intermedia phenotype [11,12]. Other mutations in the third exon of the β-globin gene have been reported to be dominant [13,14] and to cause a clinical picture of thalassemia intermedia.

The remainder of the patients carried recessive mutations, and would, from their phenotype, have been expected to have 2 thalassemia alleles. However, in these patients (10 families), only one β-thalassemia allele was identified despite sequence analysis of the β-globin gene. Based on genetic analysis, these patients would have been predicted to be thalassemia minor. Two sibs with the same phenotype were found in some families, implying a familial basis. We examined the α-globin gene status of these individuals. In 3 families, excess α-globin genes were found (Table III). These patients will be discussed separately below. In the 7 of the remaining families, a normal complement of α-globin genes was found.

The patients had a variable phenotype. Some were only mildly anemic, with mild splenomegaly, whereas others were more severely anemic, with typical end organ effects of thalassemia (bony disease, endocrinopathies, etc.). As the genetic elements responsible for the thalassemia phenotype in this group of patients may differ among the individuals studied, the variable clinical picture is expected.

α-Globin Gene Analysis

As coincident α-thalassemia is known to ameliorate the β-thalassemia phenotype [15,16] we examined for α-globin gene rearrangements (deletions or multiplications) in β-thalassemia patients with unexplained phenotypes. This included those with only one β-thalassemia allele and others in whom the clinical picture was milder than expected, considering the severity of the mutations (including patients in Groups II–V). Two individuals were found to have deletion of a single α-globin gene, but none were found to have 2 or more genes deleted (data not shown.)

Excess α-globin genes were found in 5 patients from

3 families. They were either hetero- or homozygous for the $\alpha\alpha\alpha^{anti3.7}$ configuration (data not shown).

All patients heterozygous for β-thalassemia in conjunction with excess α-globin genes (ααα/αα) had a mild clinical course. Splenomegaly was minimal to mild, as was the anemia (9.2–11.7 g Hb), with a peripheral blood smear compatible with thalassemia intermedia rather than thalassemia trait. Hb F was only mildly elevated in this group of patients (ranging from 6–11%). One patient, who was heterozygous for IVS1 nt 1 (G-A), and homozygous for a triplicated α-gene locus (ααα/αααα), had an untransfused Hb level as low as 7.0 and a modest transfusion requirement to maintain normal growth. This patient was previously described in detail [10].

The presence of ααα/αα appears to adversely affect the phenotype of thalassemia intermedia patients. Two patients of Group I, who carried −101 C-T in compound heterozygosity with a severe β-thalassemia mutation (IVS2 nt 745 C-G), had a more severe phenotype than the rest of the group (earlier age at diagnosis, the presence of thalassemic facies, and more severe anemia.)

β-Globin Haplotype Analysis in Selected Groups of Patients

Two groups of patients, those with homozygosity for IVS1 nt 6 and those homozygous for −28 A-C, showed widely disparate phenotypes. Since the severity of the disease has been shown to be associated with β-globin haplotype [17–19], haplotype analysis was performed on selected individuals.

The IVS1 nt 6 homozygotes analyzed were all found to carry the mutation in linkage to haplotype VI, except for one family in which linkage to haplotype VII was found. It has been suggested [20] that for this mutation, haplotype VII correlates with a severe and VI with a milder clinical course. The family with haplotype VII had a relatively severe phenotype. Haplotype VI linkage, however, did not uniformly correlate with a mild phenotype. As described above, this group presents a widely disparate range of severity.

Homozygosity for the mutation −28 A-C was observed to be correlated with both transfusion-dependent thalassemia major and transfusion-independent thalassemia intermedia. Haplotype analysis showed that all of the −28 A-C alleles were linked to haplotype I. Therefore, haplotype analysis was not informative as to the cause of the variable clinical condition.

DISCUSSION

Comparison With Thalassemia Major Genotypes

One of our aims was to allow for the ability to predict the severity of the thalassemia intermedia phenotype based on the genotypic analysis. Our genetic analysis revealed that 29 genotypes, comprised of 18 mutations, underlie thalassemia intermedia in Israeli patients. This marked molecular heterogeneity underlies the very wide spectrum of clinical manifestations of the disease.

Our studies show that compound heterozygosity for a silent or other mild mutation (−101 C-T, −88 C-A, and Hb Knossos, Group I) is invariably associated with mild thalassemia intermedia. Two additional genotypes, of which the present sample size is small (3 families each), have thus far been found to be associated only with mild thalassemia intermedia. These are compound heterozygosity for −28 A-C and a point mutation in the poly(A) signal, and heterozygosity for β-thalassemia in combination with 5 α-globin genes (ααα/αα). All the above genotypes, which allow for prediction of a mild phenotype, account for only 13 (22%) of the 60 families studied (Tables II and III). The remaining 47 (78%) of the thalassemia intermedia families carried genotypes which were not specific to thalassemia intermedia and were also found in thalassemia major patients in Israel (Table II) [8].

HbF Production and Thalassemia Intermedia in Israel

Elevated HbF production has been known, for some time, to ameliorate the severity of β-thalassemia. All of the patients in group V benefitted substantially from the elevation in HbF (ranging from 5.2 to 8.33 g/dl). Similarly, HbF was probably also beneficial in those individual patients of Groups II–IV who had HbF levels in the upper ranges for the group. However, elevated HbF may also reflect anemic stress [21], and is therefore not always an indicator of the presence of mild disease. Furthermore, in patients with a single thalassemia allele (Table III), mild elevation of HbF is a clinical marker for thalassemia intermedia, as opposed to thalassemia minor. This is seen in individuals of Group I, who had HbF levels above those seen with thalassemia trait.

Elevated γ-globin gene expression may be controlled by genetic elements which function in cis, such as promoter mutations or mutations in other, closely linked regulatory elements. Several β-globin gene cluster haplotypes (III, IV, IX) [17,22], associated with high HbF production, have been shown to correlate with disease severity of both β-thalassemia and sickle cell anemia [17–19]. However, these haplotypes are rare in our population, which may explain the lack of correlation between haplotypic linkage and disease severity in our study.

HbF levels are also influenced by unlinked genetic modifiers [23,24]. In our heterogenous population, there are likely to be multiple such factors, which may account for the variability in HbF and total Hb levels in patients with the same β-globin genotype (for example, groups II, IV). Both our study and others [25] have noted no correlation between HbF levels and the clinical presentation in homozygotes for IVS1 nt 6. Furthermore, in a recent study, detailed analysis of several linked elements

that might affect HbF production did not provide an explanation for the variations in HbF levels, suggesting unlinked modifiers [25]. Another recent study of heterozygotes for the IVS1 nt 6 mutation [26] reported a very variable phenotype in heterozygotes for this mutation despite similar β-globin chromosomal background (XmnI polymorphism at −158 to the $^{G}\gamma$ gene), indicating unknown ameliorating genetic determinants [26].

Interactions With α-Globin Genotype

In none of our patients did we find α-thalassemia to confer a beneficial effect on the severity of the disease. No patient was found to have only 2 (or fewer than 2) functional α-globin genes, which would be expected to be beneficial. We attribute this to the relative scarcity of α-thalassemia alleles in our communities. This is in contrast to areas such as Cyprus [15,27] or Sardinia [28], where α-thalassemia carriership is widespread. Other recent studies have also failed to find a correlation between milder disease and concurrent α-thalassemia [26] in patients with identical β-globin genotypes.

Point mutations leading to α-thalassemia are not yet fully characterized in Israel, though rare Kurdish Jewish and Arab individuals with a point mutation (AAT-AAG) in the polyadenylation signal have been described. We examined 12 of the patients whose phenotype was milder than would be expected from the genotype (including patients from Groups II–IV) comprising Jews, Arabs, and 1 Samaritan, for this point mutation. Our analysis was performed by PCR with oligonucleotide hybridization (Oron, unpublished results). In addition, using the same methodology, the Arab patients included in this sample were also analyzed for a pentanucleotide deletion in the IVS1 donor splice site of the α_2 globin gene, which has rarely been found in this population. Neither of the two point mutations were found in any of the patients.

Previous studies [29–31], including our own [10], showed the presence of 5 α-globin genes could cause a heterozygote for β-thalassemia to develop a clinical picture compatible with mild thalassemia intermedia. These patients were consistently mildly affected. In one individual with 6 α-globin genes, the clinical picture was more severe [10].

Influence of Unknown Genetic Modifiers

Accumulating evidence points to the presence of additional unlinked, some as yet unknown, genetic factors influencing the severity of the anemia [24–26,32]. In our study, the −28 A-C β-globin genes, present almost exclusively in Kurdish Jews, were all found in linkage to haplotype I. Despite this ethnic homogeneity, disease severity was very variable in −28 A-C homozygotes, suggesting the interplay of unlinked factors. Similarly, the effect of unlinked genetic modifiers is suggested by the heterogeneity of the IVS1 nt 6 homozygotes [25]; see also Table II or heterozygotes [26]. The IVS1 nt 6 mutation is widely distributed in many Mediterranean countries and is found on multiple genetic backgrounds. In Israel, it is found on two different haplotypic backgrounds (Mediterranean types VI and VII) among Moslem and Christian Arabs, Samaritans, and rarely, in Kurdish Jews, implying multiple genetic modifiers.

In 7 (21%) of the families with only one β-thalassemia allele, the molecular reason for the phenotype of thalassemia intermedia could not be determined. In these families, some of whom had two sibs with thalassemia intermedia, sequence analysis of the β-globin gene failed to reveal a second thalassemia allele. No α-globin gene rearrangement was found. These results also suggest the presence of genetic modifiers which increase the severity of what would otherwise have been thalassemia trait.

Implications for Genetic Counseling

Prediction is an important parameter in genetic analysis as it provides guidelines for genetic counseling. We analyzed a large number of families, including nearly 100 patients, in order to use the genotype/phenotype correlations for genetic counseling and other clinical decision making. In 22% of the families studied here, the identification of β-thalassemia mutations could be used to predict a mild phenotype with reasonable confidence. In 66% of the families, we would have been able to predict an individual affected with thalassemia, but we would not be able to predict the severity of its phenotype. In the remainder of the families (12%) we would have erroneously predicted thalassemia minor.

It has been suggested that genetic counseling and termination of pregnancy is justified for thalassemia intermedia [33] since in some patients, morbidity can be severe even if mortality is not high. Our results confirm the very variable and at times quite severe phenotype of thalassemia intermedia, which warrants consideration of prenatal diagnosis in selected families. However, genetic counseling based only on genotypic information is likely to be inadequate for many families. Further study to define the nature of genetic modifiers which interact with β-thalassemia will provide the basis for more accurate genetic counseling as well as enabling a better understanding of the disease process.

ACKNOWLEDGMENTS

We acknowledge the many referring physicians, including Dr. A. Abramov (Share Tzedek Hospital, Jerusalem), Prof. I. Barak and Prof. A. Berebi (Kaplan Hospital, Rehovoth), Prof. G. Cividalli and Dr. M. Aker (Hadassah University Hospital, Jerusalem), and Dr. A. Koren (Haemek Hospital, Afula). This work was supported by a grant from the Chief Scientist's Office, Ministry of Health, Israel; grant 91-00055 from the United States–

Israel Binational Science Foundation; NIH grant 7R37 DK13983-06; and the Rochlin Foundation.

REFERENCES

1. Baysal E, Carver M: The β- and δ-thalassemia repository. Hemoglobin 13:213, 1995.
2. Modell B, Berdoukas V: "The Clinical Approach to Thalassemia." London: Grune & Stratton, Ltd., 1984.
3. Weatherall DJ, Clegg JB: "The Thalassemia Syndromes." Oxford: Blackwell Scientific Publications, 1981.
4. Rund D, Filon D, Strauss N, Rachmilewitz EA, Oppenheim A: Mean corpuscular volume of heterozygotes for β-thalassemia correlates with the severity of mutations. Blood 79:238, 1992.
5. Goossens M, Kan YW: DNA analysis in the diagnosis of hemoglobin disorders. Methods Enzymol 76:805, 1981.
6. Miller SA, Dykes DD, Polesky HF: A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16:1215, 1988.
7. Rund D, Cohen T, Filon D, Dowling CE, Warren TC, Barak I, Rachmilewitz EA, Kazazian HH, Oppenheim A: Evolution of a genetic disease in an ethnic isolate: β-thalassemia in the Jews of Kurdistan. Proc Natl Acad Sci USA 88:310, 1991.
8. Filon D, Oron V, Krichevski S, Shaag A, Shaag Y, Warran TC, Goldfarb A, Shneor Y, Koren A, Aker M, Abramov A, Rachmilewitz EA, Rund D, Kazazian HH, Oppenheim A: Diversity of β-globin mutations in Israeli ethnic groups reflects recent historic events. Am J Hum Genet 54:836, 1994.
9. Orkin SH, Kazazian HH, Antonarakis SE, Goff SC, Boehm CD, Sexton JP, Waber PG, Giardina PJ: Linkage of β-thalassaemia mutations and β-globin gene polymorphisms with DNA polymorphisms in human β-globin gene cluster. Nature 296:627, 1982.
10. Oron V, Filon D, Oppenheim A, Rund D: Severe thalassemia intermedia caused by interaction of homozygosity for α-globin gene triplication with heterozygosity for β^o thalassemia. Br J Haematol 86:377, 1994.
11. de Castro C, Devlin B, Fleenor D, Lee M, Kaufman R: A novel β-globin mutation, βDurham-NC [β114 Leu-Pro], produces a dominant thalassemia-like phenotype. Blood 83:1109, 1994.
12. Murru S, Poddie D, Sciarratta G, Agosti S, Baffico M, Melevendi C, Piratsu M, Cao A: A novel β globin structural mutant, Hb Brescia (β114 Leu-Pro), causing a severe β-thalassemia intermedia phenotype. Hum Mutat 1:124, 1992.
13. Thein SL, Hesketh C, Taylor P, Temperley IJ, Hutchinson RM, Old JM, Wood WG, Clegg JB, Weatherall DJ: Molecular basis for dominantly inherited inclusion body β-thalassemia. Proc Natl Acad Sci USA 87:3924, 1990.
14. Kazazian HH, Dowling CE, Hurwitz RL, Coleman M, Stopeck A, Adams JG: Dominant thalassemia-like phenotypes associated with mutations in exon 3 of the β-globin gene. Blood 79:3014, 1992.
15. Wainscoat J, Kanavis E, Wood W, Letsky E, Huehns E, Marsh G, Higgs D, Clegg J, Weatherall D: Thalassemia intermedia in Cyprus: The interaction of alpha and beta thalassemia. Br J Haematol 53:411, 1983.
16. Wainscoat JS, Old JM, Weatherall DJ: The molecular basis for the diversity of beta-thalassemia in Cypriots. Lancet ii:1235, 1983.
17. Labie D, Pagnier J, Lapoumeroulie C, Rouabhi F, Dunda BO, Chardin P, Beldjord C, Wajcman H, Fabry ME, Nagel RL: Common haplotype dependency of high $^G\gamma$-globin gene expression and high Hb F levels in β-thalassemia and sickle cell anemia patients. Proc Natl Acad Sci USA 82:2111, 1985.
18. Thein SL, Wainscoat JS, Sampietro M, Old JM, Cappellini D, Fiorelli G, Modell B, Weatherall DJ: Association of thalassaemia intermedia with a β-globin gene haplotype. Br J Haematol 65:367, 1987.
19. Thein SL, Hesketh C, Wallace RB, Weatherall DJ: The molecular basis of thalassaemia major and thalassaemia intermedia in Asian Indians: Applications to prenatal diagnosis. Br J Haematol 70:225, 1988.
20. Chehab FF, Der Kaloustian V, Khouri FP, Deeb SS, Kan YW: The molecular basis of β-thalassemia in Lebanon: Application to prenatal diagnosis. Blood 69:1141, 1987.
21. Rochette J, Craig J, Thein S: Fetal hemoglobin levels in adults. Blood Rev 8:213, 1994.
22. Diaz-Chico J, Yang K, Stoming T, Efremov D, Kutlar A, Kutlar F, Aksoy M, Altay C, Gurgey A, Kilinc Y, Huisman T: Mild and severe β-thalassemia among homozygotes from Turkey: Identification of the types by hybridization of amplified DNA with synthetic probes. Blood 71:248, 1988.
23. Oppenheim A, Yaari A, Rund D, Rachmilewitz EA, Nathan D, Wong C, Kazazian HHJ, Miller B: Intrinsic potential for high fetal hemoglobin production in a Druze family with β-thalassemia intermedia is due to an unlinked genetic factor. Hum Genet 86:175, 1990.
24. Thein S, Sampietro M, Rohde K, Rochette J, Weatherall D, Lathrop G, Demenais F: Detection of a major gene for heterocellular hereditary persistance of fetal hemoglobin after accounting for genetic modifiers. Am J Human Genet 54:214, 1994.
25. Efremov D, Dimovsky A, Baysal E, Ye Z, Adekile A, Ribbeiro M, Schiliro G, Altay C, Gurgey A, Efremov G, Huisman T: Possible factors influencing the haemoglobin and fetal haemoglobin levels in patients with β-thalassaemia due to a homozygosity for the IVS-I-6 (T-C) mutation. Br J Haematol 86:824, 1994.
26. Waye J, Eng B, Paterson M, Wasi P, Chui D, Francombe W, Sher G, Olivieri N: Severity of β-thalassemia due to genotypes involving the IVS-1-6 (T-C) mutation. Am J Hematol 50:15, 1995.
27. Baysal E, Kleanthous M, Bozkurt G, Kyrri A, Kalogirou E, Angastiniois M, Ioannu P, Huisman T: Alpha-thalassaemia in the population of Cyprus. Br J Haematol 89:496, 1995.
28. Cao A, Rosatelli C, Galanello R: Population-based genetic screening. Curr Opin Genet Dev 1:48, 1991.
29. Galanello R, Ruggeri R, Paglietti E, Addis M, Melis M, Cao A: A family with segregating triplicated alpha loci and beta thalassemia. Blood 62:1035, 1983.
30. Camaschella C, Bertero MT, Serra A, Dall'Acqua M, Gasparini P, Trento M, Vettore L, Perona G, Saglio G, Mazza U: A benign form of thalassaemia intermedia may be determined by the interaction of triplicated alpha locus and heterozygous beta-thalassaemia. Br J Haematol 66:103, 1987.
31. Kulozik A, Thein S, Wainscoat J, Gale R, Kay L, Wood J, Weatherall D, Huehns E: Thalassaemia intermedia: Interaction of the triplicated α-globin gene arrangement and heterozygous β-thalassaemia. Br J Haematol 66:109, 1987.
32. Camaschella C, Mazza U, Roetto A, Gottardi E, Parziale A, Travi M, Fattore S, Bacchiega D, Fiorelli G, Cappellini M: Genetic interactions in thalassemia intermedia: Analysis of β mutations, α genotype, γ promoters and β-LCR hypersensitive sites 2 and 4 in Italian patients. Am J Hematol 48:82, 1995.
33. Kollia P, Voskaridou E, Rombos J, Loutradi A, Marinakis T, Fessas P, Loukopoulos D: Prenatal diagnosis of thalassemia intermedia: Is it justified? Ann NY Acad Sci 612:521, 1990.